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ORIGINAL ARTICLE

A Multivariate Analysis Model of Changes in Some Laboratory Parameters in Response to COVID-19, Diabetes, Gender, and Age

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SUMMARY

Background: **The aim of this study was to investigate changes in some laboratory parameters in response to four independent variables (COVID-19, diabetes, gender, and age) using univariate and multivariate analysis.**
Methods: **We measured WBC (neutrophil and lymphocytes), RBC and platelet counts, and hemoglobin, lactate dehydrogenase, C-reactive protein, IL-2, IL-4, and vitamin D3 levels in 30 hospitalized patients with severe COVID-19 and in 30 healthy people in terms of COVID-19. The population was divided into groups based on each of the variables of age, gender, COVID-19, and type 2 diabetes. Then they were subjected to univariate and multivariate analysis of logistic regression.**
Results: **Based on CBC data, leukocytosis (in 70% of COVID-19 patients, 61.1% of diabetic patients, and 70.9 ± 18 years old), neutrophilia (in 73.3% of patients with COVID-19, 61.1% of diabetic patients, and 66 ± 18.6 years old), neutropenia (in 6.7% of patients with COVID-19, 27.8% of diabetic patients, and 33.6 ± 12.7 years old), lymphocytosis (10% of patients with COVID-19, 33.3% of diabetic patients, and 35.4 ± 15.5 years old), and lymphocytopenia (in 76.7% of patients with COVID-19, 66.7% of diabetic patients, and 67.1 ± 18.8 years old) were observed in the population. The elderly and those with COVID-19 had significant abnormal RBC and platelet counts. Increased LDH and CRP levels and abnormal hemoglobin level were related to elderly, COVID-19, and diabetes conditions. Although the levels of IL-2 and -4 were significant in patients with COVID-19 and elderly; however, the changes were not significant in diabetic patients. Changes in serum vitamin D levels were not significant in any of the sub-groups.**
Conclusions: **We showed that leukocytosis, neutrophilia, lymphocytopenia, abnormal counts of RBCs and platelets, the elevated levels of LDH and CRP, and abnormal hemoglobin levels in blood are considered as poor prognostic factors for COVID-19.**
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KEY WORDS

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INTRODUCTION

To date, the world has experienced many outbreaks of coronavirus-related infections, including severe acute respiratory syndrome coronavirus (SARS-COV) in 2002 - 2003 and Middle East respiratory syndrome coronavirus (MERS-COV) in 2011. However, none of them had ever become a global pandemic. As of 7 January 2020 the Chinese authorities announced that they had identified a new type of COV and named it 2019 novel Coronavirus (2019-nCoV), which was changed to "The Corona Virus Disease 2019" (COVID-19) by the WHO on 12 January [1,2]. After that, the outbreaks of COVID-19 spread around the world in a short time and WHO officially declared it as a pandemic disease so that by the end of December 2020, there have been about 62,662,181 confirmed patients with COVID-19 globally and death toll has climbed to 1,460,223 [3]. Due to the impact of this disease on the health of communities, many studies have tried to determine the effective factors in 2019-nCoV pathogenicity, prevalence, mortality, and morbidity rates. However, many studies have reported the results of their research only by considering the effect of COVID-19 on the variables being measured, and they did not include other risk factors that the patient may have had at the time of the studies. Many researchers have attempted to determine the effects of SARS-CoV-2 on the human immune system and cytokine profile; however, most of them have not usually considered the medical history and other underlying diseases of the patients. That is a crucial matter, especially since the earlier studies illustrated that the risk of severe COVID-19 might be elevated in patients with cancer, chronic liver and kidney disease, COPD (chronic obstructive pulmonary disease), hypertension, pregnancy, weakened immune system, obesity, heart failure, coronary artery disease, cardiomyopathies, Sickle cell disease, and diabetes mellitus diseases [4].

Diabetes mellitus (DM) is a chronic metabolic disorder distinguished by hyperglycemia [5]. Severity and mortality of COVID-19 were nearly three-fold higher in diabetic patients than others [6,7]. Additionally, comorbidities analysis of diabetic patients with COVID-19 has showed that the prevalence of hypertension (56.9%), cardiovascular disease (20.9%), and cerebrovascular disease (7.8%) was increased compared to those without diabetes (28.8%, 11.1%, and 1.3%, respectively) [8]. Furthermore, DM can alter the cell-mediated immunity (such as chemotaxis, phagocytosis, and cytokine profile), resulting in exaggerated production of cytokines and chronic inflammation [9]. A combination of diabetes condition (especially in uncontrolled diabetes) with COVID-19 may dysregulate the immune system more than ever. As a result, the current study was performed to evaluate the changes in some laboratory parameters of COVID-19 patients in regard to diabetes status of the study population.

On the other hand, cytokine storm is a phenomenon that can damage to lung tissue and causes other complications such as pneumonia, acute respiratory syndrome (ARDS), respiratory failure, shock, other organ failure and even death [10]. Earlier studies revealed that an effective immune response to viral infection (such as COVs) and designing a vaccine for them are highly dependent on the balance between the responses of Th1 (pro-inflammatory cytokines) and Th2 (anti-inflammatory cytokines) [11-13]. Based on the studies on SARS-COV, MERS-COV, and SARS-CoV-2 infections, a "Cytokine storm" of $CD4^+$ T cells is detected by producing cytokines such as IFN-γ, TNF-α, and IL-2 for Th1 cells and IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 for Th2 cells [14]. From an immunological perspective, the present study focused on the roles of IL-2 and IL-4 in COVID-19 patients. IL-2 and IL-4 are crucial for the development of T cells into Th1 and Th2 cells, respectively [15]. Some previous studies have shown that IL-2 and IFN-γ (secreted by Th1) in critical patients were significantly lower than in severe patients. Since IL-2 is necessary for T cell proliferation and differentiation, the IL-2 signaling pathway can be effective in decreasing $CD8^+$ T cells in the critical patients [16]. Although the role of IL-4 (secreted by Th2) in defense against viruses is unclear, it has been shown that the IL-4 - related signaling pathways can affect lung tissue in acute respiratory distress syndrome (ARDS) [17]. In this study, the levels of IL-2 and IL-4 were measured in patients with severe COVID-19 to determine their changes in this type of ARDS.

In addition, studies have shown that there is a close link between vitamin D deficiency, diabetes, and some infections (such as HIV and COVID-19), which may be due to immune system involvement in all three [18-21]. Vitamin D converts to its functional form (1,25-dihydroxvitamin D) by 1-α-hydroxylase enzyme (CYP27-B1), which is present in the kidneys, keratinocytes, and immune cells (macrophages, dendritic cells, B and T lymphocytes) [18]. In general, vitamin D shifts the adaptive immune system from Th1 toward Th2 responses [22]. Th1 cell proliferation is inhibited by 1,25-dihydroxvitamin D, resulting in lowered production of IFN-γ and IL-2 [23]. As a result, dendritic cells presented less antigen and the proliferation of T cells were inhibited [24]. In connection with COVID-19, several studies showed that vitamin D supplementation had reduced the severity of the disease [25,26].

Considering all the above mentioned, the aim of this study was to investigate some immunological, biochemical and hematological parameters in response to four independent variables (COVID-19, diabetes, gender, and age) in the study population using univariate and multivariate analysis.

MATERIALS AND METHODS

Subjects and research design

In this cross-sectional study, participants were recruited from those hospitalized in ICU of Nour Afshar Hospi-

tal, Tehran, Iran. A total of 60 subjects, 30 affected by COVID-19 and a population of 30 healthy individuals were selected, and ethics approval was obtained from the human research ethics committees of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1399.179). Informed consent form was signed by all subjects. Then, samples of about 10 mL whole blood were collected in both clot tubes (7 mL) and EDTA treated tubes (3 mL) for the following tests.

All cases of COVID-19 were diagnosed based on a positive SARS-CoV-2 real-time RT-PCR test and having type-2 diabetes was defined by the patient's medical record prior admission and having a fasting blood sugar (FBS) above 110 mg/dL at the time of admission. Independent variables include patient's status of diabetes and COVID-19 along with their demographic characteristic including age and gender and the variables including WBC differential count and the levels of CRP, LDH, vitamin D, IL-2, and IL-4. The present study assessed the association of each independent variable with each the dependent variables and then evaluated the combined effect of independent variables on each of the dependent variables.

Clinical laboratory test

Cell counting and other hematological parameters were measured using a hemocytometer (Sysmex Xp3000, France). Lactate dehydrogenase (LDH) as a predictor blood biochemistry test for COVID-19 and CRP as an immunological test were carried out by LDH kit (Pars Azmoon, Tehran, Iran) and CRP kit (Biorex Diagnostic Ltd., Antrim, United Kingdom), respectively. 25-Hydroxy-vitamin D levels were measured in serum by a competitive chemiluminescence immunoassay according to the manufacturer's Euroimmun Kit (Nima Pooyesh Teb Company, Tehran, Iran). The cytokine levels of IL-2 and IL-4 were measured by ELISA kit (Invitrogen; Thermo Fisher Scientific, Inc., USA).

Statistical analysis

Descriptive statistics was carried out and data was represented as mean ± SD for quantitative variables and as frequency percentage for qualitative variables. For regression analyses, we considered blood factors as response variable and age, gender, diabetic and COVID-19 status as independent variables. Then, the statistically significant data from univariate analysis of each response variable underwent a multivariate analysis to determine the combined effect of those independent variables on that dependent variable. Odds ratio (OR) and 95% confidence intervals (CI) were reported for significant associations, and the area under the curve (AUC) was also reported to assess the predictive power for the logistic regression model. Chi-squared test was used to evaluate the association between blood type and other variables. In all tests, p-values < 0.05 were considered statistically significant. All statistical analysis was performed in R software using pROC, brglm, and VGM packages.

RESULTS

Characteristics of subjects

The basic information about participants is summarized in Table 1. Accordingly, a total of 60 subjects were enrolled, 30 affected by COVID-19 (gender: 10 women (52.6%) and 20 men (48.8%); age: 66.26 ± 19.76 years old; blood typing: AB^+ = 13.33%, AB^- = 3.33%, A^+ = 6.66, A^- = 6.66%, B^+ = 26.66, B^- = 6.66, O^+ = 33.33, and O^- = 6.66%) and a population of 30 healthy individuals (gender: 9 women (47.4%) and 21 men (51.2%); age: 32.86 ± 12.19 years old; blood typing: AB^+ = 6.66%, AB^- = 0%, A^+ = 30, A^- = 0%, B^+ = 16.66, B^- = 3.33, O^+ = 36.66, and O^- = 6.66%). While there were no significant differences in terms of gender and blood typing between both groups (p = 0.781 and p = 0.273, respectively), the age of individuals showed a significant difference (p = < 0.001).

Clinical laboratory studies

Blood cell changes in response to four independent variables (COVID-19, diabetes, gender, and age)

The data analysis of blood cells was shown in Table 2. In a univariate analysis, leukocytosis was significantly more prevalent in 30% of individuals with COVID-19 (non-adjusted odd ratio (NAOR) = 26.9, 95% CI = 3.13 - infinity, p = 0.028), 38.9% of type 2 diabetic patients (NAOR = 10.6, 95% CI = 2.43 - 94, p = 0.004), and older age (mean age: 70.9 ± 18; NAOR= 1.05, 95% CI = 1.01 - 1.1, p = 0.01). Moreover, the association between these variables was not significant in a multivariate regression model (Table 2; AUC = 0.88). In univariate analysis of red blood cells, the odds of abnormal cell count increased in 83.3% of patients with a positive COVID-19 infection (NAOR = 17.5, 95% CI = 5.3 - 82.7, p = < 0.001) and advanced age (mean age: 60.1 ± 23.5; NAOR = 0.96, 95% CI = 0.92 - 0.98, p = 0.001); however, COVID-19 was the only significant independent variable after a multivariate analysis in the subsets (adjusted odd ratio (AOR) = 14.2, 95% CI = 2.9 - 148, p = 0.003; AUC = 0.82). Similarly, a positive COVID-19 infection (NAOR = 35.98, 95% CI = 4.26 - infinity, p = 0.016) and advanced age (mean age: 69.6 ± 19.7; NAOR = 0.96, 95% CI = 0.92 - 0.98, p = 0.006) decreased the possibility of normal platelet count upon univariate analysis, but none of which were significant in a multivariate analysis (AUC = 0.84).

Based on univariate analysis of WBCs differential counting, the incidence of neutrophilia increased in the elderly (NAOR = 0.94, 95% CI = 0.92 - 0.97, p = < 0.001), in 73.3% of patients with COVID-19 (NAOR = 42.2, 95% CI = 10.6 - 169, p = < 0.001), and in 61.1% of diabetic patients (NAOR = 3.01, 95% CI = 1.02 - 8.88, p = 0.045), in which COVID-19 was the only significant variable after analyzing by multiple regression. In addition, 76.7% of patients with COVID-19 (NAOR = 45.9, 95% CI = 11.3 - 187, p = < 0.001), 66.7% of diabetic patients (NAOR = 3.35, 95% CI = 1.12 - 10.04, p = 0.0305), and advanced age (mean age: 67.1 ± 18.8;

Table 1. The basic information of the study participants.

Characteristics		Groups		p-value
		control	case	
Gender	female	n = 9 (47.4%)	n = 10 (52.6%)	0.781
	male	n = 21 (51.2%)	n = 20 (48.8%)	
Age (year)		32.866 ± 12.190	66.266 ± 19.760	< 0.001
Blood type	AB^+	n = 2 (6.66%)	n = 4 (13.33%)	0.273
	AB^-	n = 0 (0%)	n = 1 (3.33%)	
	A^+	n = 9 (30%)	n = 2 (6.66%)	
	A^-	n = 0 (0%)	n = 1 (3.33%)	
	B^+	n = 5 (16.66%)	n = 8 (26.66%)	
	B^-	n = 1 (3.33%)	n = 2 (6.66%)	
	O^+	n = 11 (36.66%)	n = 10 (33.33%)	
	O^-	n = 2 (6.66%)	n = 2 (6.66%)	
	Total	n = 30	n = 30	

Table 2. Blood cell outcomes at data cutoff in the study population.

Response variables	Independent variables		Cutoff	n (%) Mean ± SD	* Univariable		** Multivariable		AUC
					NAOR (95% CI)	p-value	AOR (95% CI)	p-value	
WBC (Reference range = 4 - 11)	COVID-19	+	4 - 11	21 (70)	26.9 3.13 - Inf	0.028	15.4 0.99 - Inf	0.095	0.88
			> 11	9 (30)					
		–	4 - 11	30 (100)	Ref.		Ref.		
			> 11	0 (0.0)					
	Diabetes mellitus	+	4 - 11	11 (61.1)	10.6 2.43 - 94	0.004	5.3 1.03 - 63.5	0.059	
			> 11	7 (38.9)					
		–	4 - 11	40 (95.2)	Ref.		Ref.		
			> 11	2 (4.8)					
	Gender	M	4 - 11	35 (85.4)	0.86 0.2 - 4.76	0.843	-	-	
			> 11	6 (14.6)					
		F	4 - 11	16 (84.2)	Ref.				
			> 11	3 (15.8)					
	Age		4 - 11	45.8 ± 22.3	1.05 1.01 - 1.1	0.01	1.00 0.95 - 1.06	0.971	
			> 11	70.9 ± 18					
RBC (Reference range = 4.70 - 6.10	COVID-19	+	normal	5 (16.7)	Ref.	< 0.001	Ref.	0.003	0.82
			abnormal	25 (83.3)					
		–	normal	24 (80)	17.5 5.3 - 82.7		14.2 2.9 - 148		
			abnormal	6 (20)					
	Diabetes mellitus	+	normal	6 (33.3)	Ref.	0.151	-	-	
			abnormal	12 (66.7)					
		–	normal	23 (54.8)	2.3 0.77 - 8.1				
			abnormal	19 (45.2)					
	Gender	M	normal	23 (56.1)	2.64 0.89 - 9.2	0.095	-	-	
			abnormal	18 (43.9)					
		F	normal	6 (31.6)	Ref.				

Table 2. Blood cell outcomes at data cutoff in the study population (continued).

Response variables	Independent variables		Cutoff	n (%) Mean ± SD	* Univariable		** Multivariable		AUC
					NAOR (95% CI)	p-value	AOR (95% CI)	p-value	
	Gender	F	abnormal	13 (68.4)	Ref.	0.095	-	-	0.82
	Age		normal	38.3 ± 17.7	0.96 0.92 - 0.98	0.001	0.96 - 1.04	0.824	
			abnormal	60.1 ± 23.5					
PLT (Reference range = 140 - 450)	COVID-19	+	normal	19 (63.3)	Ref.	0.016	Ref.	0.058	0.84
			abnormal	11 (36.7)					
		–	normal	30 (100)	35.98 4.26 - Inf		23.37 1.8 - Inf		
			abnormal	0					
	Diabetes mellitus	+	normal	14 (77.8)	0.68 0.2 - 3.01	0.576	-	-	
			abnormal	4 (22.2)					
		–	normal	35 (83.3)	Ref.				
			abnormal	7 (16.7)					
	Gender	M	normal	33 (80.5)	0.84 0.15 - 3.1	0.803	-	-	
			abnormal	8 (19.5)					
		F	normal	16 (84.2)	Ref.				
			abnormal	3 (15.8)					
	Age		normal	45.1 ± 21.9	0.96 0.92 - 0.98	0.006	0.99 0.95 - 1.02	0.518	
			abnormal	69.6 ± 19.7					

NAOR = 1.06, 95% CI = 1.03 - 1.01, p = < 0.001) were more likely to have lymphocytopenia. In multivariate analysis, COVID-19 was the only significant factor (AOR = 25.02, 95% CI = 4.6 - 137, p = 0.001).

Changes in protein laboratory parameters in response to four independent variables (COVID-19, diabetes, gender, and age)

Based on univariate analysis in Table 3, the levels of LDH were increased in 90% of positive COVID-19 patients (NAOR = 24.6, 95% CI = 6.8 - 153, p = < 0.001) and elderly (mean age: 61.1 ± 22.1; NAOR = 1.06, 95% CI = 1.03 - 1.11, p = < 0.001). Among diabetic participants, LDH was significantly elevated in 77.8% of diabetic patients (NAOR = 3.5, 95% CI = 1.1 - 15.4, p = 0.0466). Moreover, the results of multivariate analysis showed that COVID-19 was the only significant independent variable (AOR = 9.83, 95% CI = 2.1 - 97.1, p = 0.009; AUC = 0.87).

In addition, elevated CRP level was also related to 80% of COVID-19 patients (NAOR = 11.8, 95% CI = 3.8 - 48.9, p = 0.001), 83.3% of diabetic patients (NAOR = 7.11, 95% CI = 2.1 - 39.2, p = 0.004), and older age (mean age: 59.6 ± 23; NAOR = 1.04, 95% CI = 1.02 - 1.08, p = 0.002) based on univariate analysis in Table 3, but none remained significant after a multivariate analysis (calculated AUC was 0.82).

In univariate analysis of hemoglobin, abnormality was significantly associated with COVID-19 (NAOR = 3.92, 95% CI = 1.4 - 12.8, p = 0.0136), DM (NAOR = 5.36, 95% CI = 1.72 - 21.37, p = 0.006), and advanced age (mean age: 59.7 ± 22; NAOR = 0.97, 95% CI = 0.94 - 0.99, p = 0.007), whereas, there was no meaningful relationship among them in a multivariate analysis (AUC = 0.75).

Changes in serum level of vitamin-D in response to four independent variables (COVID-19, diabetes, gender, and age)

In univariate analysis of vitamin-D3, no significant differences were observed between this variable with COVID-19 (p = 0.001), DM (p = 0.545), gender (p = 0.545), and age (p = 0.234), which are shown in Table 3.

Changes in serum cytokines (IL-2 and IL-4) in response to four independent variables (COVID-19, diabetes, gender, and age)

Based on Table 3, IL-2 was significantly increased in 70% of patients with COVID-19 (NAOR = 25.8, 95% CI = 6.65 - 231, p = < 0.001) and in older participants (mean age: 63.3 ± 21.6; NAOR = 1.04, 95% CI = 1.02 - 1.08, p = 0.001). After a multivariate analysis, COVID-19 was the only significant independent factor (AOR = 24.1, 95% CI = 4.2 - 349, p = 0.001; AUC = 0.834). Sixty percent of patients with COVID-19 (NAOR = 90.3, 95% CI = 10.8 - infinity, p = 0.002) and older participants (mean age: 62.6 ± 21; NAOR = 1.03, 95% CI

Table 3. The data of blood proteins, vitamin D and interleukins at data cutoff in the studied population.

Response variables	Independent variables		Cutoff	N (%) or mean ± SD	* Univariable		** Multivariable		AUC
					NAOR (95% CI)	p-value	AOR (95% CI)	p-value	
LDH (Reference range = 140 - 300	COVID-19	+	140 - 300	3 (10)	24.6 6.8 - 153	< 0.001	9.83 2.1 - 97.1	0.009	0.87
			> 300	27 (90)					
		–	140 - 300	23 (76.7)	Ref		Ref		
			> 300	7 (23.3)					
	Diabetes Mellitus	+	140 - 300	4 (22.2)	3.5 1.1 - 15.4	0.0466	1.01 0.15 - 6.4	0.92	
			> 300	14 (77.8)					
		–	140 - 300	22 (52.4)	Ref.		Ref		
			> 300	20 (47.6)					
	Gender	M	140 - 300	17 (41.5)	1.27 0.42 - 3.82	0.672	-	-	
			> 300	24 (58.5)					
		F	140 - 300	9 (47.4)	Ref.				
			> 300	10 (52.6)					
	Age		140 - 300	34.5 ± 15.4	1.06 1.03 - 1.11	< 0.001	1.03 0.98 - 1.08	0.241	
			> 300	61.1 ± 22.1					
CRP more than 5 compared with less than 5	COVID-19	+	positive	24 (80)	11.8 3.8 - 48.9	< 0.001	8.3 1.75 - 68.7	0.147	0.82
			negative	6 (20)					
		–	positive	7 (23.3)	Ref.		Ref.		
			negative	23 (76.7)					
	Diabetes Mellitus	+	positive	15 (83.3)	7.11 2.1 - 39.2	0.004	4.27 1.01 - 28.6	0.0641	
			negative	3 (16.7)					
		–	positive	16 (38.1)	Ref.		Ref.		
			negative	26 (61.9)					
	Gender	M	positive	20 (48.8)	0.7 0.22 - 2.06	0.532	-	-	
			negative	21 (51.2)					
		F	positive	11 (57.9)	Ref.				
			negative	8 (42.1)					
	Age		positive	59.6 ± 23	1.04 1.02 - 1.08	0.002	1.00 0.96 - 1.04	0.995	
			negative	38.8 ± 18.8					
Hemoglobin	COVID-19	+	normal	12 (40)	Ref.	0.0136	Ref.	0.54	0.75
			abnormal	18 (60)					
		–	normal	22 (73.3)	3.92 1.4 - 12.8		1.63 0.33 - 7.9		
			abnormal	8 (26.7)					
	Diabetes Mellitus	+	normal	5 (28.7)	Ref.	0.006	Ref.	0.085	
			abnormal	13 (31)					
		–	normal	29 (69)	5.36 1.72 - 21.37		3.19 0.91 - 14		
			abnormal	13 (31)					
	Gender	M	normal	25 (61)	0.58 0.19 - 1.72	0.339	-	-	
			abnormal	16 (39)					
		F	normal	9 (47.4)	Ref.				
			abnormal	10 (52.6)					
	Age		normal	41.8 ± 22.1	0.97 0.94 - 0.99	0.007	0.98 0.95 - 1.02	0.347	
			abnormal	59.7 ± 22					

= 1.01 - 1.07, p = 0.009) had significantly elevated levels of IL-4 in comparison with others. In multivariate analysis, COVID-19 was the only significant factor in both IL-2 (AOR = 24.1, 95% CI = 4.2 - 349, p = 0.001) and IL-4 (AOR = 197.2, 95% CI = 15 - infinity, p = 0.001). The AUCs for IL-2 and IL-4 in this model were 0.834 and 0.886, respectively.

DISCUSSION

Various factors affect the pathogenicity, mortality and mortality rate of 2019-nCoV which should be considered during the study of patients. Accordingly, in the present study, an attempt was made to analyze the changes in laboratory parameters by considering the four variables of age, gender, diabetes, and COVID-19. Other similar studies have also used similar methods to identify multiple predictive and risk factors affecting COVID-19 [27-30].

This study revealed that leukocytosis was observed in 30% patients with COVID-19. Previous studies had proposed that leukocytosis in severely affected patients with COVID-19 had a poor prognosis, while leukocytopenia was associated with a better prognosis [31,32]. For instance, Sayad et al. revealed that leukocytosis was more prevalent in severe COVID-19 patients (with the prevalence of 11.4%) than patients with mild to moderate disease (with the prevalence of 4.8%) [32]. Since all patients in the present study had been selected among patients with severe COVID-19, admitted to ICU, the results confirmed the earlier results.

On the other hand, some researchers had shown that there was a significant association between high peripheral WBC counts (as a marker of inflammation) and some inflammatory conditions that put individuals at increased risk of COVID-19, including type 2 diabetes, coronary artery disease (CAD), and diabetes micro- and macro-vascular complications [33-36]. An earlier study had suggested that increased WBC count in type 2 diabetes, even within the normal range, along with unfavorable results of metabolic tests (including higher blood pressure, BMI, HbA1c, fasting plasma glucose, LDL cholesterol, triglycerides, and urinary albumin excretion, but lower HDL cholesterol) could induce both macro- and micro-vascular complications in patients with type 2 diabetes [36]. Herein, we showed that 38.9% of type 2 diabetic patients had an elevated WBC count and the prevalence of leukocytosis was higher in diabetic patients compared to healthy people. Since diabetic patients are more prone to infection diseases compared to non-diabetic individuals, the effect of COVID-19 and diabetes on WBC count was also subjected to a multivariate analysis in which only COVID-19 was significant.

In addition, the studies examining the association between high WBC count and age have shown that mortality risk was elevated with increasing WBC count in elderly [37]. Based on the Nilsson et al. study, an elevated WBC count in elderly was a useful predictor of survival in 75-year-olds and that was related to cardiovascular mortality in both genders and non-cardiovascular mortality in women [37]. Similarly, we showed that leukocytosis was present in patients 70.9 ± 18 years old in the recent study.

Additionally, the differential white blood cell counts showed the presence of neutrophilia (in 73.3% of patients with COVID-19, 61.1% of diabetic patients, and 66 ± 18.6 years old), neutropenia (in 6.7% of patients with COVID-19, 27.8% of diabetic patients, and 33.6 ± 12.7 years old), lymphocytosis (10% of patients with COVID-19, 33.3% of diabetic patients, and 35.4 ± 15.5 years old), and lymphocytopenia (in 76.7% of patients with COVID-19, 66.7% of diabetic patients, and 67.1 ± 18.8 years old) in the study population. The results of the study showed that the incidence of neutrophilia and lymphocytopenia was higher than other conditions in elderly and in individuals with COVID-19 and diabetes. Neutrophil hyperinflammation may be a common phenomenon in many viral infections, including hepatitis, COVID-19, and influenza [38]. These results are similar to the earlier studies which revealed an elevated peripheral neutrophil-to-lymphocyte ratio in relation to a poor prognosis in patients with severe COVID-19 [39].

In addition to WBC, most patients with severe COVID-19 had abnormal RBC count and hemoglobin level in the present study. Although aging also reduces RBC counts, a multivariate analysis showed that the abnormality in the number of these cells was due to COVID-19 and not the age of the participants. Due to the role of RBC in oxygen transport, changes in this cell may contribute to the worsening of hypoxia in critically ill patients. Previous studies have suggested that the virus can alter the protein and lipid contents of RBC membranes [40]. Also, the virus enters RBCs by the Spike glycoprotein and can affect the function of hemoglobin after binding to the 1-beta chains of hemoglobin and sometimes interfering with the heme group [41,42]. However, Thomas et al. reported that there were no changes in RBC count, hematocrit, or mean corpuscular hemoglobin concentration, but these hematological parameters were significantly more abnormal in patients with COVID-19 than in healthy people in the recent study [40]. Similarly, some studies revealed that a decreased hemoglobin level is observed in COVID-19, which was more prevalent in severe COVID-19 than milder forms [32]. However, another study using artificial intelligence on the data of 53 patients hospitalized in China found that mildly increased alanine aminotransferase, the presence of myalgias, and elevated hemoglobin level were related to a poorer outcomes [43]. Although the main clinical symptom in patients with COVID-19 is shortness of breath, about 60 - 70% of hospitalized patients usually develop coagulation disorders such as thrombocytopenia, hypercoagulation, disseminated intravascular coagulation (DIC), and venous thrombosis (VT) [44]. Thus, the platelet count was measured and, based on data of 36.7% of our patients with

severe COVID-19, they were abnormal. In according with previous studies, a mild thrombocytopenia can occur in 5 to 41.7% of patients depending on the severity of COVID-19 and poor prognosis. Thrombocytopenia is more prevalent in COVID-19 patients when they are elderly and men, and have higher APACHE II score, lower total neutrophil and lymphocyte counts, higher CRP and a lower PaO2/FiO2 ratio [45]. Although age and COVID-19 were both significant in univariate analysis, neither was significant after multivariate analysis in a recent study.

In parallel with previous studies, elevated levels of LDH and CRP were observed in patients with diabetes and COVID-19, which indicate a more severe inflammatory response and tissue damage in these diseases [46]. In relation to age and lactate dehydrogenase, some studies suggested that elderly patients with diseases involving the immune system (such as multiple myeloma) had a poor prognosis when lactate dehydrogenase was high [47]. However, due to the small sample size in this study, we could not examine the effect of this predictor factor and only adjusted the population in terms of participants' age. Based on data, 61.1 ± 22.1 year old patients had LDH values above 300 U/L in a recent study. On the other hand, a cytokine storm occurs in COVID-19 patients, which results in a change in the cytokine profile. Some studies revealed that the levels of IL-1B, IL-1RA, IL-6, IL-7, IL-8, IL-9, IL-10, fibroblast growth factor (FGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFNγ, granulocyte colony-stimulating factor (G-CSF), interferon-γ-inducible protein (IP10), monocyte chemoattractant protein (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) were increased, among which IL-2, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A, and TNF-α were higher in severe patients [48]. IL-2, as a T cell growth factor, is released by activated $CD4^+$ T cells and $CD8^+$ T cells. Based on data, IL-2 at high levels can increase $CD4^+$ T cells and active $CD8^+$ T cells, while these cells are inhibited by low levels of IL-2. Shi et al. showed that IL-2R, JAK1, and STAT5 were downregulated in PBMC of common, severe, and critical patients with COVID-19; however, the plasma level of IL-2 was elevated in severe patients and decreased in critical patients. In addition, the percentage of $CD8^+$ T cells were lower in COVID-19 patients compared to healthy individuals and critical patients had lower $CD8^+$ T cell counts than severe patients. They proposed that this reduction of $CD8^+$ T cells may be related to IL-2 and its role in the JAK1-STAT5 signaling pathway [16]. Similarly, we showed increased IL-2 levels in 70% of patients with severe COVID-19. Because of the age-related changes in human cytokine responses, all results were age-adjusted. On the other side, IL-4 as an anti-inflammatory cytokine is involved in various Th2-induced signaling pathways and can shift the immune response from Th1 to Th2 by inhibiting Th1-mediated responses [15]. Based on the Renu et al. study, the roles of IL-4 in COVID-19 associated inflammation pathway depends on the molecular weight of the spike proteins of the coronavirus so that the proteins with more than 70 kDa of weight trigger the cellular immunity via the Th1 cell pathway (activating IFN-γ) while the proteins with less than 70 kDa activated humoral immunity via the Th2 cell pathway (activating IL-4) [15]. Paula et al. reported that there was an increase in IL-4 expression and lung damage in patients with SARS-CoV-2 in which the contribution of Th2 responses was higher than that Th1/Th17 response [17]. Similar to some previous studies, we revealed that about 60% of severe COVID-19 patients had high serum levels of IL-4. Moreover, some studies demonstrated that in type 2 DM some changes were induced in the levels of pro- and anti-inflammatory T cell cytokines; however, there was no significant association between IL-2/IL-4 levels and type 2 diabetes in present study [49,50].

Contrary to the some epidemiological studies which showed a significant inverse correlation with vitamin D deficiency and incidence and severity of COVID-19, no significant association was observed among vitamin D deficiency, and any of the independent variables in our patients with severe COVID-19 [20,25,51]. Studies examining the association between vitamin D and viral infections have shown conflicting results because of differences in methodology, demographics, vitamin D levels, VDR mutations, and supplement dosages in these studies. However, most meta-analytic studies have shown significant ecological correlations between vitamin D levels and incidence and mortality of COVID-19. In addition, they reported such indirect association between vitamin D deficiency and conditions such as diabetes and hypertension which in themselves associate with severity of COVID-19 [51].

CONCLUSION

In this study, some laboratory parameters in response to four independent variables (COVID-19, diabetes, gender, and age) were investigated using univariate and multivariate analysis in severe COVID-19 patients. Due to the significant effect of age on all tests (except vitamin D), the study population was adjusted based on age. Although univariate analysis showed that many parameters (WBCs, neutrophils, lymphocytes, LDH, CRP, and Hb) were affected by both diabetes and COVID-19 statues, only COVID-19 remained significant after multivariate analysis. Other dependent variables (RBCs, platelets, IL-2, and IL-4) were significant only in patients with COVID-19. Based on CBC results, we revealed that leukocytosis, neutrophilia, lymphocytopenia, and abnormal counts of RBCs and platelets can be considered as a poor prognostic factor for COVID-19. In addition, elevated LDH and CRP proteins and abnormal hemoglobin levels in blood are additional poor prognostic factors for COVID-19. Furthermore, 70% of severe COVID-19 patients had an elevated IL-2 level

and 60% had an increased IL-4 in this study. However, there was no significant association between vitamin D3 and the independent variables. It seems that a repetition of this study with a larger sample size can provide more reliable results because in our study population only 23.3% of COVID-19 patients and 16.7% of diabetic patients had severe vitamin D deficiency.

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Declaration of Interest:
There is no conflict of interest among authors.

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